

WHAT IS CLAIMED IS:

1 1. A zinc finger protein that binds to a target site, wherein the target site
2 has a nucleotide sequence as specified in Table 3 or 4.

1 2. The zinc finger protein of claim 1, comprising at least one finger of the
2 C2H2 class of zinc fingers.

1 3. The zinc finger protein according to claim 2, wherein the target site is
2 one of the nucleotide sequences in a row of Table 3 or 4 and positions -1 to +6 in at least one
3 of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in
4 the row.

1 4. The zinc finger protein according to claim 3, wherein positions -1 to
2 +6 in each of the three zinc fingers are occupied by first, second and third segments of seven
3 contiguous amino acids as specified in a row of Table 3.

1 5. The zinc finger protein according to claim 4, wherein the segments
2 have the amino acid sequences specified for one of the zinc finger proteins listed in Table 3,
3 wherein the zinc finger protein is selected from the group consisting of BVO 13A, EP10A,
4 GATA82Z7678, HBV 3, HP38 4A, HUM 17A, HUM 19A, MTS 5A, MX1E, PDF 5A, RAT
5 24A, SAN 16A, USX 3A, VEGF 1, VEGF 1*3, VEGF 1A, VEGF 1B, VEGF 1C, VEGF 1D,
6 VG 10A, VG 1B, VG 4A, VG 8A, VOP 28A-2, VOP 30A-4, VOP 32A-6, VOP 32B-7, VOP
7 35A-10, ZEN-7A 1, VOP 29A-3, VOP 32C, VOP 32D, VOP 32E, VOP 32F, VOP 32G,
8 VOP 32H, VOP 32I and VOP 32J.

1 6. The zinc finger protein according to claim 2, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in at least one of the six zinc fingers
3 is occupied by a segment of seven contiguous amino acids as specified in Table 4.

1 7. The zinc finger protein according to claim 2, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are
3 occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 8. The zinc finger protein according to claim 7, wherein the segments
2 have the amino acid sequences specified for a zinc finger protein selected from the group
3 consisting of BVO 10A-9A, BVO 12A-11B and BVO 14B-13A as listed in Table 4.

9. The zinc finger protein according to claim 1, wherein the zinc finger protein is a fusion protein comprising a regulatory domain.

10. The zinc finger protein according to claim 9, wherein the fusion protein comprises a plurality of regulatory domains.

11. The zinc finger protein according to claim 9, wherein the regulatory domain is an activation domain.

12. The zinc finger protein according to claim 11, wherein the activation domain is selected from the group consisting of (a) VP16, (b) p65, and (c) functional fragments of (a) and (b).

13. The zinc finger protein according to claim 9, wherein the regulatory domain is a repressor domain.

14. The zinc finger protein according to claim 13, wherein the repressor domain is selected from the group consisting of (a) KRAB, (b) methyl binding domain protein 2B, (c) v-ErbA repressor domain, and (d) functional fragments of (a), (b) and (c).

15. A zinc finger protein that binds to a target site having a nucleotide sequence as specified in Table 3 or 4 whereby the zinc finger protein can modulate angiogenesis when introduced into an animal having a genome comprising a VEGF gene comprising the target site.

16. The zinc finger protein of claim 15, comprising at least three fingers of the C₂H₂ class of zinc fingers.

17. The zinc finger protein according to claim 16, wherein the target site is one of the nucleotide sequences in a row of Table 3 or 4 and positions -1 to +6 in at least one of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in the row.

18. The zinc finger protein according to claim 17, wherein positions -1 to +6 in each of the three zinc fingers are occupied by first, second and third segments of seven contiguous amino acids as specified in a row of Table 3.

1 19. The zinc finger protein according to claim 16, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in at least one of the six zinc fingers
3 is occupied by a segment of seven contiguous amino acids as specified in Table 4.

1 20. The zinc finger protein according to claim 19, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are
3 occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 21. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger according to claim 1.

1 22. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger protein according to claim 4.

1 23. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger protein according to claim 7.

1 24. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger protein according to claim 9.

1 25. A method for modulating expression of a VEGF gene, the method
2 comprising contacting a target site of a nucleic acid within a cell with a zinc finger protein,
3 wherein the target site has a nucleotide sequence as specified in Table 3 or 4 and binding of
4 the zinc finger protein to the target site modulates expression of the VEGF gene in the cell.

1 26. The method according claim 25, wherein the expression of a plurality
2 of splice variants of the VEGF gene is modulated.

1 27. The method according to claim 25, wherein a plurality of target sites
2 are contacted with a plurality of zinc finger proteins and each zinc finger protein binds to a
3 distinct target site.

1 28. The method according to claim 27, wherein each of the plurality of
2 zinc finger proteins is a fusion protein.

1 29. The method according to claim 28, wherein each of the zinc finger
2 proteins is a fusion protein comprising a regulatory domain.

30. The method according to claim 29, wherein each zinc finger protein is fused to a different regulatory domain.

31. The method according to claim 25, wherein the zinc finger protein comprises at least three fingers of the C₂H₂ class of zinc fingers.

32. The method according to claim 31, wherein positions -1 to +6 in each of the three zinc fingers are occupied by first, second and third segments of seven contiguous amino acids as specified in a row of Table 3.

33. The method according to claim 31, wherein the zinc finger protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

34. The method according to claim 25, wherein the zinc finger protein is a fusion protein comprising a regulatory domain.

35. The method according to claim 34, wherein the method further comprises administering the zinc finger protein in combination with a delivery vehicle.

36. The method according to claim 34, wherein the method further comprises administering a nucleic acid encoding the zinc finger protein into the cell.

37. The method according to claim 36, wherein administering comprises delivering the nucleic acid into the cell in a naked form.

38. The method according to claim 36, wherein the nucleic acid is contained within an expression vector and is operably linked to a promoter, and administering comprises delivering the vector into the cell.

39. The method according to claim 38, wherein the expression vector is a viral expression vector.

40. The method according to claim 39, wherein the expression vector is selected from the group consisting of a retroviral expression vector, an adenoviral expression vector, and an AAV expression vector.

1 41. The method according to claim 38, wherein the promoter is an
2 inducible promoter.

1 42. The method according to claim 34, wherein regulatory domain
2 comprises an activation domain and binding of the zinc finger protein to the target site
3 activates transcription of the VEGF gene in the cell.

1 43. The method according to claim 42, wherein the cell is a population of
2 cells.

1 44. The method according to claim 43, wherein activation of VEGF
2 transcription activates angiogenesis in the population of cells.

1 45. The method according to claim 44, wherein the population of cells is a
2 cell culture.

1 46. The method according to claim 44, wherein the population of cells are
2 in a mammalian subject.

1 47. The method according to claim 36, wherein the zinc finger protein or
2 zinc finger protein nucleic acid are administered in an amount effective to treat a disease or
3 injury.

1 48. The method according to claim 47, wherein the disease or injury is
2 selected from the group consisting of atherosclerosis, ischemia and arthritis.

1 49. The method according to claim 47, wherein the subject has a wound
2 and the amount administered is effective to treat the wound.

1 50. The method according to claim 47, wherein the subject has an ulcer
2 and the amount administered is effective to treat the ulcer.

1 51. The method according to claim 42, wherein activation of VEGF
2 transcription activates lymphogenesis in the population of cells.

1 52. The method according to claim 42, wherein activation of VEGF
2 transcription activates myelopoiesis in the population of cells.

53. The method according to claim 42, wherein the activation domain is selected from the group consisting of (a) VP16, (b) p65, (c) functional fragments of (a) and (b).

54. The method according to claim 34, wherein the regulatory domain is a repressor domain and binding of the zinc finger protein to the target site represses transcription of the VEGF gene in the cell.

55. The method according to claim 54, wherein the cell is a population of cells.

56. The method according to claim 55, wherein repression of VEGF transcription represses angiogenesis in the population of cells.

57. The method according to claim 55, wherein the population of cells is a cell culture.

58. The method according to claim 55, wherein the population of cells are in a mammalian subject.

59. The method according to claim 58, wherein the zinc finger protein or zinc finger protein nucleic acid are administered in an amount effective to treat a disease or injury.

60. The method according to claim 59, wherein the disease is a tumor.

61. The method according to claim 54, wherein the repressor domain is selected from the group consisting of (a) KRAB, (b) methyl binding domain protein 2B, (c) v-ErbA repressor domain, and (d) functional fragments of (a), (b) and (c).

62. The method according to claim 25, wherein the target site is located in a single type of VEGF gene, and binding of the zinc finger protein to the target site modulates expression of the single VEGF gene in the cell.

63. The method according to claim 25, wherein the target site is located in a plurality of different types of VEGF genes, and binding of the zinc finger protein to the target site modulates expression of the plurality of VEGF genes.

1 64. The method according to claim 63, wherein the target site comprises a
2 nucleotide sequence bound by a protein selected from the group consisting of EP10A,
3 GATA82Z678, HBV 3, HP38 4A, HUM 17A, MTS 5A, PDF 5A, USX 3A, VEGF 1,
4 VEGF1*3, VEGF 1A, VG 10A, VG 1B, VG 4A, VG8A, VOP28A-2, VOP 30A-4, and ZEN-
5 7A 1.

1 65. The method according to claim 64, wherein the target site is the
2 nucleotide sequence recognized by VOP 28A-2.

1 66. The method of according to claim 64, wherein the target site is the
2 nucleotide sequence recognized by VOP 30A-4.

1 67. A method for modulating angiogenesis comprising introducing a zinc
2 finger protein into an animal having a genome comprising a target site within a VEGF gene,
3 whereby the zinc finger protein binds to the target site and thereby modulates angiogenesis in
4 the animal.

1 68. The method according to claim 67, wherein the modulation of
2 angiogenesis comprises inhibition of new blood vessel formation.

1 69. The method according to claim 67, wherein modulation of
2 angiogenesis comprises stimulation of new blood vessel formation.

1 70. The method according to claim 69, wherein the blood vessels are
2 nonhyperpermeable.

1 71. The method according to claim 67, wherein the zinc finger protein
2 binds to a target site specified in Table 3 or 4.

1 72. The method according to claim 71, wherein positions -1 to +6 in each
2 of three zinc fingers are occupied by first, second and third segments of seven contiguous
3 amino acids as specified in a row of Table 3.

1 73. The method according to claim 71, wherein the zinc finger protein
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 74. The method according to claim 67, wherein the target site is present in
2 a plurality of VEGF genes, whereby the zinc finger protein binds to the target site in the
3 plurality of genes, thereby modulating expression of the plurality of VEGF genes.

1 75. The method according to claim 67, wherein introducing comprises
2 introducing a plurality of zinc finger proteins into the animal, each zinc finger protein binding
3 to a different target site in the same gene.

1 76. The method according to claim 75, wherein each of the zinc finger
2 proteins is a fusion protein comprising a regulatory domain.

1 77. The method according to claim 76, wherein each zinc finger protein is
2 fused to a different regulatory domain.

1 78. A method of treating ischemia, comprising administering a zinc finger
2 protein that binds to a target site specified in Table 3 or 4 into an animal having ischemia,
3 wherein the zinc finger protein is administered in an amount effective to treat ischemia.

1 79. The method of claim 78, wherein the animal has a genome comprising
2 a VEGF gene comprising the target site and the zinc finger protein binds to the target site.

1 80. The method according to claim 79, wherein the zinc finger protein
2 comprises at least three fingers of the C₂H₂ class of zinc fingers.

1 81. The method according to claim 80, wherein positions -1 to +6 in each
2 of the three zinc fingers are occupied by first, second and third segments of seven contiguous
3 amino acids as specified in a row of Table 3.

1 82. The method according to claim 80, wherein the zinc finger protein
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 83. A method for screening for a modulator of expression of a VEGF gene,
2 the method comprising:

3 (a) contacting a test cell with a zinc finger protein and a test agent,
4 wherein the zinc finger protein comprises at least one zinc finger that binds to a target site,
5 the target site having a nucleotide sequence as specified in Table 3 or 4;

(b) comparing the level of expression of the VEGF gene in the test cell with a baseline level, a statistically significant difference in the level of expression in the test cell relative to the baseline level indicating that the test agent is a potential modulator of VEGF gene expression.

84. The method of claim 83, wherein the zinc finger is a fusion protein comprising an activation domain, and a lower level of expression in the test cell relative to the baseline level indicates that the test agent is a repressor of the VEGF gene.

85. The method of claim 83, wherein the zinc finger protein is a fusion protein comprising a repressor domain, and an increased level of expression in the test cell relative to the baseline level indicates that the test agent is an activator of the VEGF gene.

86. A pharmaceutical composition comprising a nucleic acid according to claim 14 operably linked to a regulatory sequence and a pharmaceutically acceptable carrier or diluent, wherein the regulatory sequence allows for expression of the nucleic acid in a cell.

87. The pharmaceutical composition according to claim 86, wherein the nucleic acid is contained in an expression vector.

88. The pharmaceutical composition according to claim 87, wherein the expression vector is a viral expression vector.

89. The pharmaceutical composition according to claim 88, wherein the expression vector is selected from the group consisting of a retroviral expression vector, an adenoviral expression vector, and an AAV expression vector.

90. A pharmaceutical composition comprising a zinc finger protein according to claim 1 and a pharmaceutically acceptable carrier or diluent.

91. A zinc finger protein comprising a plurality of zinc fingers, wherein at least one of the plurality of zinc fingers is occupied by a segment of seven contiguous amino acids as specified in a row of Table 3 or 4.

92. The zinc finger protein of claim 91, wherein the zinc finger protein is a three finger zinc finger protein and the at least one zinc finger is occupied by a segment of seven contiguous amino acids as specified in a row of Table 3.

93. The zinc finger protein of claim 92, wherein at least two of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in a row of Table 3.

94. The zinc finger protein of claim 93, wherein all three of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in a row of Table 3.

95. The zinc finger protein of claim 91, wherein the zinc finger protein is a six finger zinc finger protein and the at least one zinc finger is occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

96. The zinc finger protein of claim 95, wherein at least three of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

97. The zinc finger protein of claim 96, wherein all six of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

98. A method for treating a wound comprising introducing a zinc finger protein into an animal having a genome comprising a target site within a VEGF gene, whereby the zinc finger protein binds to the target site, such binding accelerating healing of the wound.